CANADIAN JOURNAL OF RESEARCH

VOLUME 27 FEBRUARY, 1949 NUMBER 1

— SECTION C —

BOTANICAL SCIENCES

Contents

Page

Studies on Steam Sterilization of Soils. II. Some Factors Affecting Minimum Sterilization Requirements—*F. J. Simpson and J. D. Newton* - - - - - - - - - - 1

NATIONAL RESEARCH COUNCIL
OTTAWA, CANADA

CANADIAN JOURNAL OF RESEARCH

The *Canadian Journal of Research* is issued in six sections, as follows:

A. Physical Sciences	D. Zoological Sciences
B. Chemical Sciences	E. Medical Sciences
C. Botanical Sciences	F. Technology

For the present, Sections A, C, D, and E are to be issued six times annually, and Sections B and F, twelve times annually, each under separate cover, with separate pagination.

The *Canadian Journal of Research* is published by the National Research Council of Canada under authority of the Chairman of the Committee of the Privy Council on Scientific and Industrial Research. The *Canadian Journal of Research* is edited by a joint Editorial Board consisting of members of the National Research Council of Canada, the Royal Society of Canada, and the Chemical Institute of Canada.

Sections B and F of the *Canadian Journal of Research* have been chosen by the Chemical Institute of Canada as its medium of publication for scientific papers.

EDITORIAL BOARD

Representing
NATIONAL RESEARCH COUNCIL

DR. A. R. GORDON, (*Chairman*),
Head, Department of Chemistry,
University of Toronto, Toronto.

DR. C. H. BEST,
The Banting and Best Department
of Medical Research,
University of Toronto, Toronto.

DR. ROBERT NEWTON,
President, University of Alberta,
Edmonton, Alta.

DR. G. H. HENDERSON,
Professor of Mathematical
Physics,
Dalhousie University, Halifax.

Representing
ROYAL SOCIETY OF CANADA

DR. A. NORMAN SHAW,
Chairman, Department of Physics
McGill University, Montreal.

DR. J. W. T. SPINKS,
Department of Chemistry,
University of Saskatchewan,
Saskatoon.

Section III

DR. E. HORNE CRAIGIE,
Department of Zoology,
University of Toronto,
Toronto.

DR. H. S. JACKSON,
Head, Department of Botany,
University of Toronto, Toronto.

Section V

Ex officio

DR. LÉO MARION, Editor-in-Chief,
Division of Chemistry,
National Research Laboratories,
Ottawa.

DR. H. H. SAUNDERSON,
Director, Division of
Information Services,
National Research Laboratories,
Ottawa.

Representing
THE CHEMICAL INSTITUTE OF CANADA

DR. R. V. V. NICHOLLS,
Associate Professor of Chemistry,
McGill University,
Montreal.

EDITORIAL COMMITTEE

Editor-in-Chief,	DR. LÉO MARION	Editor, Section D,	DR. E. HORNE CRAIGIE
Editor, Section A,	DR. A. NORMAN SHAW	Editor, Section E,	DR. J. B. COLLIP
Editor, Section B,	DR. J. W. T. SPINKS DR. R. V. V. NICHOLLS	Editor, Section F,	DR. J. A. ANDERSON DR. R. V. V. NICHOLLS DR. A. NORMAN SHAW
Editor, Section C,	DR. H. S. JACKSON		

Manuscripts should be addressed:

Editor-in-Chief,
Canadian Journal of Research,
National Research Council, Ottawa, Canada.

Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 27, SEC. C. FEBRUARY, 1949 NUMBER 1

STUDIES ON STEAM STERILIZATION OF SOILS

II. SOME FACTORS AFFECTING MINIMUM STERILIZATION REQUIREMENTS[1]

BY F. J. SIMPSON[2] AND J. D. NEWTON[3]

Abstract

For steam sterilization of 50-gm. to 1000-gm. samples of three Alberta loam soils and a peat, the required length of treatment increased with the weight and depth of sample. The peat and black soils, high in organic matter, required longer treatments than the brown or gray soils, low in organic matter. However, identical depths of different air-dry soils were found to reach 120° C. in the same time. Moistening large samples prior to autoclaving greatly reduced the time required to reach 120° C. and to obtain sterility, but had no similar effect on small samples. A moisture content of 75% water holding capacity was optimum, reducing the 180 min. required to reach 120° C. in 6 in. of air-dry peat to 80 min. Autoclaving samples in a moist condition, however, resulted in larger increases in base exchangeable ammonia than autoclaving in an air-dry condition. Generally, minimum single treatments at 15 p.s.i. gauge produced less ammonia than at 12 p.s.i. gauge and single treatments produced less ammonia than intermittent treatments. A slight reduction in nitrate on autoclaving was observed.

Introduction

This investigation is part of a broad study of inter-relationships between soil microflora, soil fertility, and plant diseases. To obtain close control over the microflora, a sterile soil was desired, but one not radically changed from the original. Most investigators have used extreme treatments in their study of changes caused by heating soils, but below 200° C. the majority of the changes appear to be comparatively small (2, 8, 9, 10, 13, 16). It was therefore felt that determination of the minimum steam treatments required to achieve sterility and a measurement of the resulting alterations in the soils would be worthwhile.

Coleman, Lint, and Kopeloff (4) investigated the possibility of sterilizing soils without radical alterations, but they did not achieve sterility with their methods. They found that the application of dry heat (80° C.) to moist soil resulted in a greater destruction of bacteria and less chemical change than

[1] *Manuscript received August 18, 1948.*

Contribution from the Department of Soils, University of Alberta, Edmonton, Alta., with financial assistance from the National Research Council of Canada.

[2] *Graduate Assistant in Soils, University of Alberta. Present address: Division of Applied Biology, National Research Council, Ottawa.*

[3] *Professor of Soils, University of Alberta.*

[The December issue of Section C (Can. J. Research, C, 26 : 501-587. 1948) was issued March 3, 1949.]

the application of various chemicals under pressure, vacuum, and in combination with heat. More recently, sterilizing soils by means of gaseous ethylene oxide has been found (6, 15) to result in a low degree of chemical change. The present investigation was confined to treating dry and moist soils in a laboratory autoclave.

Materials and Methods

Three loam soils representing the brown, black, and gray wooded soil zones of Alberta were used for this study, in addition to a peat obtained from west of Edmonton. The soils were air-dried, large lumps were broken, and extraneous materials were removed by passing the soils through an eight mesh sieve. The coarse peat was moderately ground in an attrition type soil grinder. They were then stored in large galvanized cans.

To classify the soils as to texture, a mechanical analysis was conducted by the hydrometer method outlined by Bouyoucos (3) and the results are described in Fig. 1. The Edmonton black was the heaviest of the three loams, and

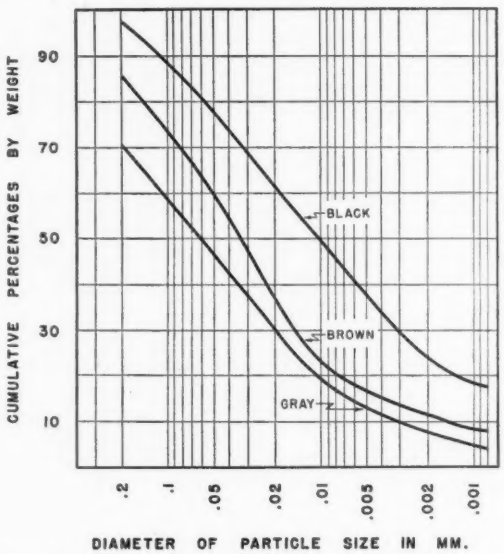

FIG. 1. *Mechanical analyses of Edmonton black, Vauxhall brown, and Breton gray wooded soils.*

although the Vauxhall brown and the Breton gray were somewhat alike, the latter contained more sand and less silt. The soils were found on analysis (Table I) to vary in fertility and organic matter content, but the pH was found to be fairly uniform.

TABLE I

COMPOSITION OF SOILS

(Percentage by weight, water-free basis)

Soil	Organic matter	Nitrogen	Hygroscopic moisture	Total water capacity	pH
Gray	2.8	0.16	4	54	6.2
Brown	3.5	0.20	3	57	7.0
Black	6.2	0.50	9	79	7.2
Peat	92.6	2.42	6	310	6.6

Samples of 50, 100, 400, and 1000 gm. of soil on a water-free basis were respectively treated in 300, 500, 1000, and 2000 ml. Erlenmeyer flasks. Similarly, 50, 100, and 250 gm. of peat were treated in 500, 1000, and 2000 ml. Erlenmeyer flasks. A Bramhall Deane autoclave fitted with a thermometer in the discharge line was used for treating the samples, and operated according to techniques described by Underwood (18). The flasks were placed in the autoclave on their sides to permit a more efficient removal of air from the flask and soil.

The treatments consisted of autoclaving the samples at 12 or 15 p.s.i. gauge steam pressure. The exposures were timed from the moment the desired pressure was reached. Single and daily consecutive treatments were given. The soils were treated at three different moisture levels; namely, air-dry and 50% and 100% water holding capacity. All samples except those treated in an air-dry condition were weighed and brought to 50% water holding capacity seven days prior to sterilization and incubated at room temperature. It was reasoned that this procedure would bring the soil flora to an active state and promote the germination of spores and cysts. The water content of the samples was checked immediately before autoclaving, and those to be treated at 100% water holding capacity were brought to this condition. After sterilization, the air-dry samples were moistened with sterile water. All samples were then incubated for 12 days at room temperature to allow any survivors to multiply and thus simplify detection.

Sterility was checked by plating in Petri dishes for preliminary and confirming runs. The Spray culture plate technique (17) was used to test for anaerobes. Fred and Waksman's (5) sodium caseinate agar and glucose nutrient agar were used. Sterile water was added to the flasks of soil and shaken, then 1 ml. of the mixture was transferred directly to the plate.

Experimental

The minimum single periods of exposure to steam at 12 and 15 p.s.i. gauge pressure, required to obtain sterility, are shown in Figs. 2 and 3. The data obtained for intermittent treatments are given in Tables II and III. The

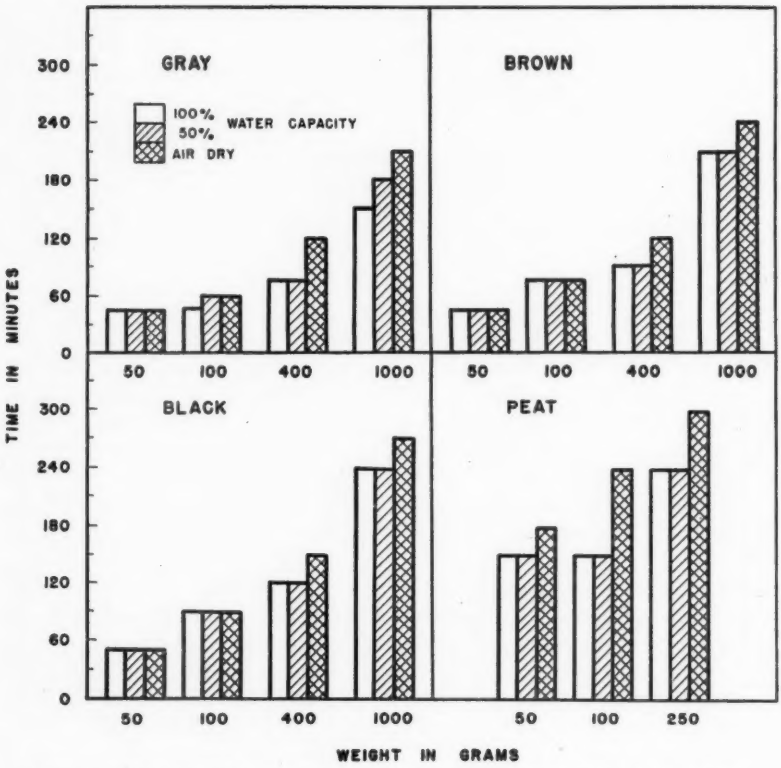

FIG. 2. *Time required to steam sterilize soils in a single treatment at 12 p.s.i. gauge.*

times given for intermittent treatments represent the length of each exposure required to sterilize samples when autoclaved on two successive days.

It is evident that an increase in the weight of sample with a corresponding increase in depth necessitated longer periods of exposure to obtain sterility. In addition, the different soils had different sterilization requirements. The difficulty with which different soils were sterilized appears to be correlated with organic matter content and texture. The gray loam, with the least amount of organic matter and coarsest texture, required the shortest treatment. The brown and black loams, with larger amounts of organic matter and finer textures, required longer periods of treatment. The peat, almost entirely composed of organic matter, required the severest steaming to attain sterility.

Incubating and autoclaving in a moist condition decreased the time required to sterilize large samples, but had no effect on small samples. The reduction was most evident in the peat, where it reduced the time required by as much as 30 and 90 min. In the main, moisture levels of 50 and 100% water holding capacity appeared to have an equal effect.

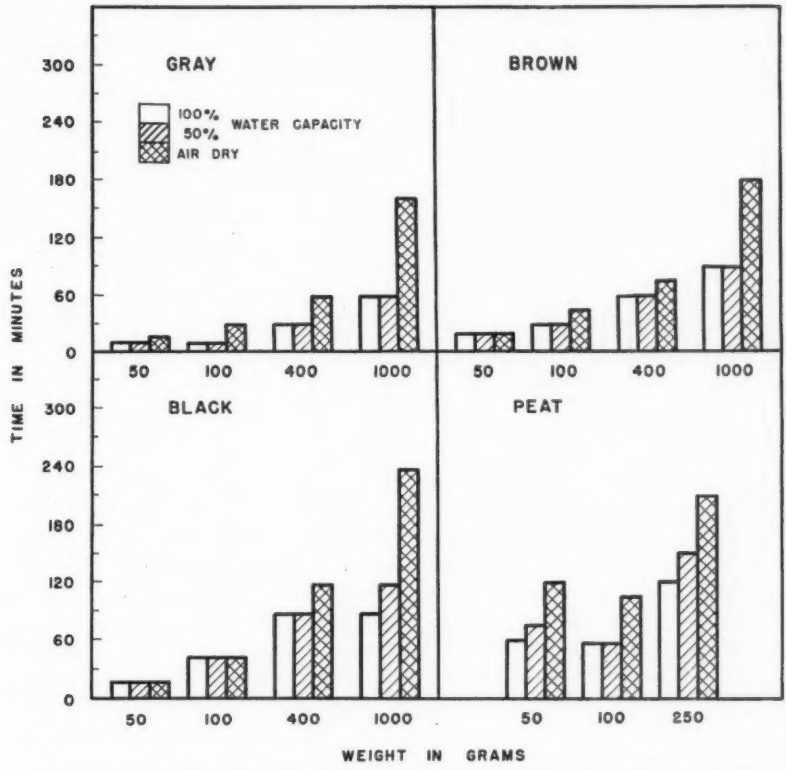

FIG. 3. *Time required to steam sterilize soils in a single treatment at 15 p.s.i. gauge.*

The effect of moisture may be explained by a more heat susceptible flora resulting from incubation in a moist condition prior to sterilization. However, the observation that incubation of 50- and 100-gm. samples usually did not result in a reduction of the time required to sterilize, indicates that some other factor may be of greater practical importance. The trends with respect to moisture, organic matter, and texture makes one suspect that the conductivity of the soils (14) may be the important limiting factor. If the limiting factor were steam penetration, a high moisture content would be detrimental. Water, having a specific heat about four times that of soil, would require a greater steam input to raise it to the same temperature and its presence would likely impede the penetration of steam. With small samples where the depth of soil was insignificant, steam penetration and conduction were either non-limiting or of equal efficiency. With greater depths, the presence of moisture resulted in greater efficiency than that given by steam penetration in the air-dry samples.

TABLE II

TIME REQUIRED TO STERILIZE SOILS BY STEAM AT 12 P.S.I. GAUGE IN TWO INTERMITTENT TREATMENTS

Soil	Size of sample, gm.	Length of each individual treatment, min.		
		Moisture, % water capacity		
		Air-dry	50	100
Gray	50	30	30	30
	100	45	45	30
	400	75	—	—
	1000	150	120	90
Brown	50	30	30	30
	100	60	60	60
	400	90	75	75
	1000	150	120	120
Black	50	30	30	30
	100	75	75	75
	400	90	90	90
	1000	210	120	120
Peat	50	120	90	90
	100	135	90	75
	250	240	180	180

TABLE III

TIME REQUIRED TO STERILIZE SOILS BY STEAM AT 15 P.S.I. GAUGE IN TWO INTERMITTENT TREATMENTS

Soil	Size of sample, gm.	Length of each individual treatment, min.		
		Moisture, % water capacity		
		Air-dry	50	100
Gray	50	—	—	—
	100	15	—	—
	400	45	—	—
	1000	90	60	60
Brown	50	15	15	15
	100	20	20	20
	400	60	45	45
	1000	150	—	—
Black	50	15	15	15
	100	30	30	30
	400	90	75	—
	1000	210	90	—
Peat	50	60	45	45
	100	60	30	30
	250	150	90	90

To check the effect of moisture on the sterilization process, copper–constantan thermocouples were inserted in the center of two inches of peat contained in a 2000 ml. beaker. Moisture levels of air-dry, 25, 50, 75, and 100% water capacity were studied at 15 p.s.i. gauge steam pressure. The results obtained (Table IV) indicate that moisture has a profound influence on the rate of heat penetration and that the optimum moisture content is around 75% water capacity.

TABLE IV

TIME REQUIRED FOR THE CENTER OF A 2 IN. DEPTH OF PEAT TO REACH 120° C.

(15 p.s.i. gauge steam pressure)

% water capacity	Air-dry	25	50	75	100
Time in minutes	50	30	20	15	25

The same moisture levels were used in a second test. The thermocouples were placed at one inch intervals beginning one-half inch below the surface in six inches of peat contained in a 2000 ml. beaker. Glass insulated wire was used and supported at the proper depths by threading through small holes bored in a three-eighths inch square stick. Readings were taken at two minute intervals for the first half hour, at five minute intervals during the second half hour, and at 10 min. intervals thereafter. These data are described in Fig. 4. Each gradient represents the temperatures of the soil atmosphere recorded at a specified time expressed in minutes from the moment that 15 p.s.i. gauge pressure was reached.

The gradients for the air-dry soil indicated a rapid initial rise in temperature one-half inch below the surface and one-half inch above the bottom of the beaker, but a slower and more regular rise in the center of the sample. As the steam penetrated, there was an abrupt rise in temperature with each individual point reached. By the time the steam penetrated the entire sample, the peat at the bottom had reached a fairly high temperature as a result of conduction. It required 180 min. for the peat to reach a uniform temperature of 120° C.

In moist samples, the temperatures at the extremes of the peat rose much more slowly. This would be expected because of the higher specific heat of water. The temperatures at the central points remained almost stationary until heat was conducted to them when they rose very quickly. Again, a moisture content of 75% water holding capacity appeared about optimum. With this moisture content, six inches of peat reached a uniform temperature of 120° C. in 80 min. Great difficulty was experienced in placing the thermocouples in the peat at 100% water holding capacity. The results obtained were not considered reliable and therefore are not given. Generally, 90 min. were required to raise the temperature of six inches of peat at 100% water holding capacity to 120° C.

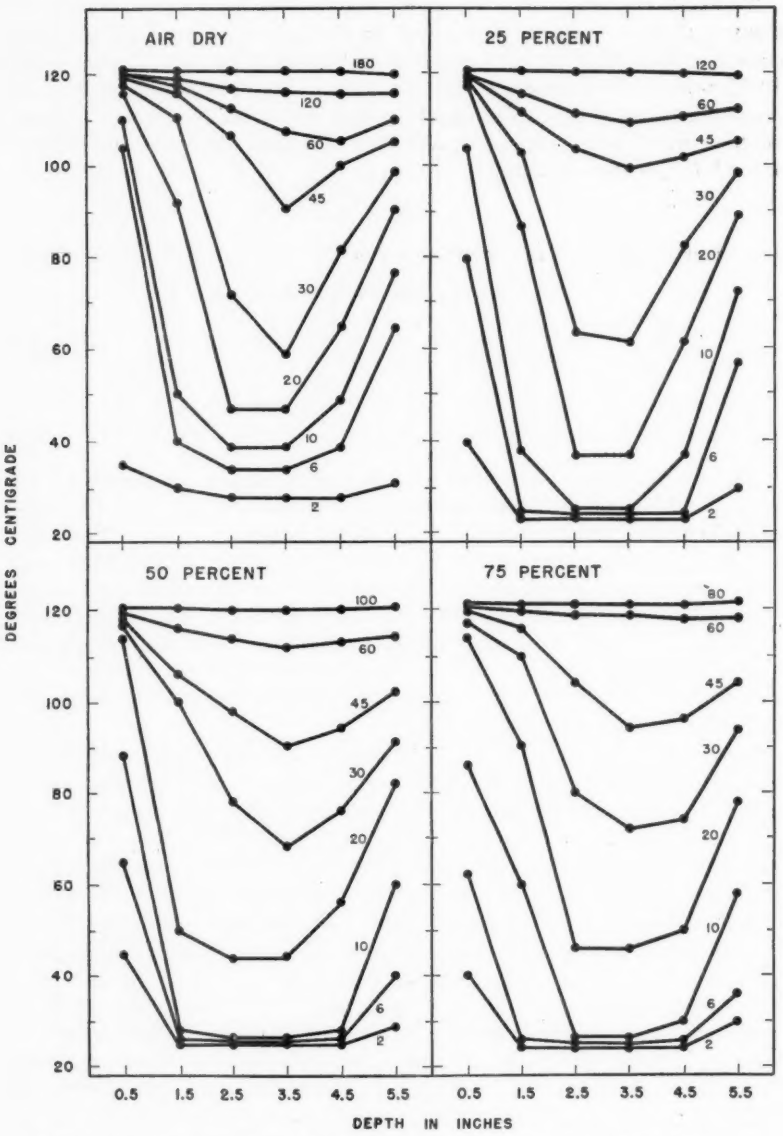

FIG. 4. *Temperature gradients at various times (minutes) in six inches of peat, at different percentages of water holding capacity, treated at 15 p.s.i. gauge steam pressure.*

It should be possible to remove some of the air held in the dry peat and reduce opposition to the penetration of steam by applying a vacuum of 20 in. immediately before steaming. The rate of temperature rise in the top four inches was somewhat accelerated by this procedure. However, it did not influence the time required to reach a uniform temperature of 120° C. Applying a vacuum to the moist soils had practically no effect.

The trends obtained with the peat were also found to hold for the gray and black loams. By comparison with air-dry soil a moisture content of 75% water holding capacity reduced the time required to reach a uniform temperature of 120° C. from 170 min. to 60 min. for the six inches of gray loam, and from 180 min. to 70 min. for the black loam. In sterilizing large samples of soil where depth is important, the presence of moisture is therefore desirable for efficient and rapid sterilization.

In a study of the effect of depth, thermocouples were placed in the center of air-dry samples from one to six inches deep. The results were rather surprising (Table V). For each depth, the time required to reach 120° C. was

TABLE V

EFFECT OF DEPTH ON THE TIME (MINUTES) REQUIRED TO RAISE THE TEMPERATURE TO 120°C.

(Air-dry soil at 15 p.s.i gauge steam pressure)

Depth of sample, in.	Depth of thermocouple, in.	Gray	Brown	Black	Peat
1	0.5	20	25	25	20
2	1	50	60	60	60
3	1.5	90	90	90	90
4	2	120	110	120	120
5	2.5	150	150	150	150
6	3	170	180	180	180

essentially the same. This observation was confirmed when mixtures of sand and 2 to 64% peat in geometric progression were studied. Regardless of the amount of peat in the mixture the center of a 2 in. depth reached a temperature of 120° C. in 50 to 60 min. One would therefore expect sterilization requirements of identical depths to be quite uniform.

In the original test the soils had been measured into various flasks on the basis of weight and it was realized that the specific gravity of soils would vary according to their texture and organic matter content. Therefore sterilization requirements of the air-dry soils were checked using depths of ½, 1, and 2 in. A dried suspension of *Bacillus subtilis* spores was thoroughly mixed with the soils and the various depths measured into one liter Erlenmeyer flasks. These were treated in an upright position at 15 p.s.i. gauge steam pressure. Sterility

was determined by adding sterile glucose nutrient broth and examining smears after three days' incubation. Samples found to be sterile were incubated an additional three days before checking with a second smear.

These data (Table VI) indicate that the original relationship between the different soils still stands, but to a lesser degree. Although the rate of steam penetration was uniform with different soils, the organic matter and texture

TABLE VI

TIME (MINUTES) REQUIRED TO STERILIZE DIFFERENT DEPTHS (AIR-DRY SOIL AT 15 P.S.I. GAUGE STEAM PRESSURE)

Depth, in.	Gray	Brown	Black	Peat
0.5	95	105	105	120
1	105	120	120	150
2	150	180	225	240

probably modify the rate of penetration of heat in the soil atmosphere to the center of the soil aggregates. If so, one would expect the size of soil aggregates to have a measureable effect on sterilization requirements of the same and different soils. The size of aggregates in the soils studied did not appear to vary greatly, but there was a trend of increasing size from gray to black. It is also interesting to note that the colloid content of the soils (Fig. 1) increases from gray to black and to peat, or in the same order as difficulty of sterilization. These colloids and the organic matter may have a protective effect on the soil organisms. Conduction appears to have a limiting role not only in moist samples but also in dry samples.

Degree of Chemical Change Resulting from Minimum Steam Sterilization Treatments

The effect of moisture, pressure, and single and intermittent treatments on the degree of chemical change resulting from sterilizing 50 and 100 gm. samples for their respective minimum periods was investigated. A review of the literature concerning chemical changes induced by steaming soils indicated that production of ammonia, increase in water soluble phosphorus, and destruction of nitrates occurred (9, 13, 16). The production of ammonia has been especially noted by a number of investigators. A relatively rapid and accurate method for determining base-exchangeable ammonia (12) was selected as a means of estimating the degree of chemical change induced by various minimum sterilization treatments. In addition, some nitrate determinations were made on 1:5 water extracts by the phenol–disulphonic method modified by Harper (7). All determinations were made on duplicate samples from duplicate runs. The samples were analyzed immediately after sterilizing and cooling, simultaneously with duplicate controls.

Tables VII and VIII show that the increase in ammonia varies with the organic matter content. In addition, a higher moisture content favors larger increases in ammonia. Where, however, treating the soils at 100% water

TABLE VII

INCREASES IN BASE-EXCHANGEABLE SOIL AMMONIA INDUCED BY STEAM STERILIZATION

(50-gm. samples subjected to minimum treatments)

Treatment	Steam pressure, p.s.i. gauge	Soil moisture, % water capacity	Ammonia increases, p.p.m.			
			Gray	Brown	Black	Peat
One single	12	Air-dry	1.1	7.4	7.3	40.9
		50	5.0	12.8	11.8	43.0
		100	4.8	11.8	13.0	34.9
	15	Air-dry	1.0	4.8	4.8	21.6
		50	4.4	4.7	9.8	108.0
		100	4.8	4.8	9.9	98.1
Two intermittent	12	Air-dry	2.9	7.4	7.5	49.2
		50	2.2	12.6	14.9	115.4
		100	3.3	13.2	13.3	81.0
	15	Air-dry	—	2.0	6.4	19.0
		50	—	1.7	12.7	42.0
		100	—	2.8	12.9	38.6

TABLE VIII

INCREASES IN BASE-EXCHANGEABLE SOIL AMMONIA INDUCED BY STEAM STERILIZATION

(100-gm. samples subjected to minimum treatments)

Treatment	Steam pressure, p.s.i. gauge	Soil moisture, % water capacity	Ammonia increases, p.p.m.		
			Gray	Brown	Black
One single	12	Air-dry	2.6	1.0	10.8
		50	5.0	3.7	15.0
		100	3.0	4.1	11.9
	15	Air-dry	2.2	2.3	6.2
		50	8.7	4.1	14.0
		100	5.6	5.7	14.8
Two intermittent	12	Air-dry	6.3	1.3	10.3
		50	9.4	8.8	20.1
		100	6.5	9.1	19.8
	15	Air-dry	1.0	2.0	6.0
		50	—	5.9	8.6
		100	—	6.4	10.9

capacity so increased the sterilization efficiency as to allow a large reduction in length of treatment, less ammonia was obtained. It is interesting to note that sterilizing the soils in an air-dry condition regardless of the longer treatments required, resulted in production of less ammonia than sterilizing in a moist condition.

Minimum treatments at a steam pressure of 15 p.s.i. gauge resulted in ammonia increases smaller than or equal to those produced at 12 p.s.i. gauge (Table IX). Somewhat larger increases in ammonia were generally obtained with two intermittent treatments than with single treatments.

TABLE IX

EFFECT OF MOISTURE CONTENT AND NUMBER OF CONSECUTIVE TREATMENTS ON THE AMMONIA PRODUCED BY STEAM STERILIZATION OF THE MINERAL SOILS

Moisture, % water capacity	Number of intermittent treatments	Average ammonia increases, p.p.m.	
		12 p.s.i. gauge	15 p.s.i. gauge
Air-dry	1	5.0	3.7
	2	5.9	4.0
	3	6.7	7.8
50	1	8.8	7.3
	2	11.3	7.3
	3	11.1	—
100	1	8.0	7.6
	2	11.0	8.2
	3	13.8	—

No definite trend of increases or decreases in the nitrate-nitrogen contents of the sterilized soils were obtained (Table X) although a small loss was observed with those treated at 15 p.s.i. gauge.

TABLE X

WATER SOLUBLE NITRATE NITROGEN IN 50-GM. SAMPLES OF STERILIZED AND NONSTERILIZED SOILS

Steam pressure, p.s.i. gauge	Moisture, % water capacity	Nitrate nitrogen, p.p.m.					
		Gray		Brown		Black	
		Control	Sterilized	Control	Sterilized	Control	Sterilized
12	Air-dry	3.0	3.1	22.4	23.1	32.0	34.4
	50	3.0	3.1	22.0	18.6	42.4	38.7
	100	3.2	2.7	23.2	18.9	42.3	38.6
15	Air-dry	3.4	3.6	21.3	20.6	32.0	31.8
	50	3.7	4.0	22.3	20.0	32.8	30.0
	100	4.0	3.2	22.0	18.2	35.0	34.3

With the exception of peat, the small changes in ammonia and nitrate-nitrogen contents of the soils caused by minimum sterilization treatments were not greater than normal variations found to occur in soils under natural conditions. By autoclaving soils in very shallow layers it appears possible to achieve sterility quickly and without radical alteration. Even under ideal conditions, however, steam sterilization induces small changes. Nevertheless, the use of steam sterilized soil in a study of the inter-relationships of soil micro-organisms, plant diseases, and soil fertility appears to be warranted.

References

1. BAVER, L. D. Soil physics. John Wiley and Sons, Inc., New York. 1940.
2. BOUYOUCOS, G. J. Effect of ignition at various temperatures upon certain physical properties of soils. Soil Sci. 17 : 135-139. 1924.
3. BOUYOUCOS, G. J. Direction for making mechanical analysis of soils by the hydrometer method. Soil Sci. 42 : 225-228. 1936.
4. COLEMAN, D. A., LINT, H. C., and KOPELOFF, N. Can soil be sterilized without radical alteration? Soil Sci. 1 : 259-274. 1916.
5. FRED, E. B. and WAKSMAN, S. A. Laboratory manual of general microbiology. McGraw-Hill Book Company, Inc., New York. 1928.
6. HANSEN, N. H. and SNYDER, W. C. Gaseous sterilization of biological materials for use as culture media. Phytopathology, 37 : 369-371. 1947.
7. HARPER, H. J. Method for determination of nitrates. Ind. Eng. Chem. 16 : 180-183. 1924.
8. KELLEY, W. P. and MCGEORGE, W. The effect of heat on Hawaiian soils. Hawaii. Agr. Expt. Sta. Bull. 30. 1913.
9. KELLEY, W. P. The organic nitrogen of Hawaiian soils. II. Effects of heat on soil nitrogen. J. Am. Chem. Soc. 36 : 434-438. 1914.
10. KOPELOFF, N. and COLEMAN, D. A. A review of investigations in soil protozoa and soil sterilization. Soil Sci. 3 : 197-269. 1917.
11. MCCULLOCH, E. C. Disinfection and sterilization. Lea & Febiger, Philadelphia. 1936.
12. MCLEAN, W. and ROBINSON, G. W. A new method for the determination of ammoniacal nitrogen in soils. J. Agr. Sci. 14 : 548-554. 1924.
13. MALOWANY, S. C. and NEWTON, J. D. Studies on steam sterilization of soils. I. Some effects on physical, chemical, and biological properties. Can. J. Research, C, 25 : 189–208. 1947.
14. PATTEN, H. E. Heat transference in soils. U.S. Dept. Agr. Bur. Soils Bull. 59. 1909.
15. ROBERTS, J. I., ALLISON, L. E., PRICKETT, P. S., and RIDDLE, K. B. Preliminary studies on soil sterilization with ethylene oxide. J. Bact. 45 : 40 (Abstract). 1943.
16. SCHREINER, O. and LATHROP, E. C. The chemistry of steam-heated soils. U.S. Dept. Agr. Bur. Soils Bull. 89. 1912. *Also in* J. Am. Chem. Soc. 34 : 1242-1259. 1912.
17. SPRAY, R. B. An improved anaerobic culture dish. J. Lab. Clin. Med. 16 : 203-206. 1930.
18. UNDERWOOD, W. B. A textbook of sterilization. American Sterilizer Company. Erie, Penn. 1941.

CANADIAN JOURNAL OF RESEARCH

Notes on the Preparation of Copy

GENERAL:—Manuscripts should be typewritten, double spaced, and the **original and one extra copy** submitted. Style, arrangement, spelling, and abbreviations should conform to the usage of this Journal. Names of all simple compounds, rather than their formulae, should be used in the text. Greek letters or unusual signs should be written plainly or explained by marginal notes. Superscripts and subscripts must be legible and carefully placed. Manuscripts should be carefully checked before being submitted, to reduce the need for changes after the type has been set. If authors require changes to be made after the type is set, they will be charged for changes that are considered to be excessive. **All pages, whether text, figures, or tables, should be numbered.**

ABSTRACT:—**An abstract of not more than about 200 words, indicating the scope of the work and the principal findings, is required.**

ILLUSTRATIONS:

(i) *Line Drawings:*—All lines should be of sufficient thickness to reproduce well. Drawings should be carefully made with India ink on white drawing paper, blue tracing linen, or co-ordinate paper **ruled in blue only**; any co-ordinate lines that are to appear in the reproduction should be ruled in black ink. Paper ruled in **green, yellow, or red should not be used** unless it is desired to have all the co-ordinate lines show. Lettering and numerals should be neatly done in India ink preferably with a stencil (**do not use typewriting**) and be of such size that they will be legible and not less than one millimetre in height when reproduced in a cut three inches wide. All experimental points should be carefully drawn with instruments. Illustrations need not be more than two or three times the size of the desired reproduction, but the ratio of height to width should conform with that of the type page. **The original drawings and one set of small but clear photographic copies are to be submitted.**

(ii) *Photographs:*—Prints should be made on glossy paper, with strong contrasts; they should be trimmed to remove all extraneous material so that essential features only are shown. Photographs should be submitted **in duplicate**; if they are to be reproduced in groups, one set should be so arranged and mounted on cardboard with rubber cement; the duplicate set should be unmounted.

(iii) *General:*—**The author's name, title of paper, and figure number should be written in the lower left hand corner (outside the illustration proper) of the sheets on which the illustrations appear.** Captions should not be written on the illustrations, but typed on a separate page of the manuscript. All figures (including each figure of the plates) should be numbered consecutively from 1 up (arabic numerals). **Each figure should be referred to in the text.** If authors desire to alter a cut, they will be charged for the new cut.

TABLES:—Titles should be given for all tables, which should be numbered in Roman numerals. Column heads should be brief and textual matter in tables confined to a minimum. **Each table should be referred to in the text.**

REFERENCES:—**These should be listed alphabetically by authors' names, numbered in that order, and placed at the end of the paper.** The form of literature citation should be that used in the respective sections of this Journal. **Titles of papers should not be given in references listed in Sections A, B, E, and F, but must be given in references listed in Sections C and D.** The first page only of the references cited in papers appearing in Sections A, B, and E should be given. **All citations should be checked with the original articles.** Each citation should be referred to in the text by means of the key number; in Sections C and D the author's name and the date of publication may be included with the key number if desired.

The *Canadian Journal of Research* conforms in general with the practice outlined in the *Canadian Government Editorial Style Manual*, published by the Department of Public Printing and Stationery, Ottawa.

Reprints

Fifty reprints of each paper without covers are supplied free. Additional reprints, if required, will be supplied according to a prescribed schedule of charges. On request, covers can be furnished at cost.